Wolman's Syndrome. SOS was clinically diagnosed and treatment began between 7 and 37 days post-HSCT (mean=17 days). Patients were treated per protocol with a starting dose of 10 mg/kg/day with subsequent increases of 10 mg/kg/day until clinically therapeutic to a max of 60 mg/kg/day (exception: < 2 y/o population allowed a maximum of 100 mg/kg/day, n=1). The duration of defibrotide treatment ranged from 13 to 31 days (mean=18 days).There were no significant complications related to the defibrotide infusions or therapy. Seven of 11 (63.6%) patients showed complete response to the therapy, while 4 patients were classified as non-responders. The 100 day post-HSCT survival rate was 54.5% (6/11) for all patients. In conclusion, this study provides further evidence that defibrotide is an efficacious and safe treatment for pediatric SOS occurring after HSCT.

71

VIRUS-SPECIFIC T CELLS ENGINEERED TO CO-EXPRESS TUMOR-SPECIFIC RECEPTORS; EFFECTS IN PATIENTS WITH NEUROBLASTOMA

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Long-term survival for children with neuroblastoma remains poor despite intensification of therapy. Neuroblastoma cells express a number of potential target antigens for the immune response including GD2 and T cells engineered to express chimeric antigen receptors (CAR) for these antigens might supplement conventional therapeutics. Unfortunately, modification of primary T cells with CAR has demonstrated limited functionality and persistence in vivo, likely because the target tumor cells lack the co-stimulatory molecules required for T cell activation. We have previously shown that Epstein Barr Virus specific cytotoxic T lymphocytes (EBV-CTLs) persist and function in vivo long term, in part because they receive co-stimulation from EBV infected B lymphocytes. We reasoned that EBV-CTLs engineered to express GD2-CAR would also receive such co-stimulatory signals following engagement of their native (EBV-specific) receptors, and hence would persist longer than primary T cells expressing the same CAR. We therefore developed a Phase I clinical study to directly compare EBV CTL-CAR and T-cell CAR. We used two retroviral vectors distinguishable by a non-expressed marker sequence so that signal detected in vivo could be assigned to the CTL or the T cells. Vector use was alternated between the two cell populations. To date 3 patients received 2×10E7/m2 of each cell population, while 4 have received 1×10E8/m2 of each product. We observed no adverse effects. Even at the low cell doses used to date, signal could be detected for 6 weeks in peripheral blood. Differential quantitative PCR analysis of the signal showed that EBV-CTL CAR were present at a higher level and persisted longer than primary T cell associated CAR. Clinically there has been one mixed response and two with stable disease, while one patient treated in CR remains disease free at 12 months. 4 patients have died of progressive disease. Expressing chimeric antigen receptors in virus specific T cells appears to improve lymphocyte survival after adoptive transfer compared to CAR expression in primary T cells, and may thereby increase the therapeutic potential of these cells. It will be of interest to use this approach early after stem cell transplantation, when residual malignant cells are at their lowest level, and homeostatic signals will favor expansion of adoptively transferred engineered CTL.

72

EFFECTIVE IMMUNOTHERAPY FOR NEUROBLASTOMA REQUIRES HSCT AND T CELL TRANSFER

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We have employed a mouse model for neuroblastoma, AGN2a cells injected subcutaneously in to strain A/J mice, to demonstrate that transfection of AGN2a with CD80, CD86, CD54, and CD137L transforms this lethal cell line in to an effective cell-based vaccine (AGN2a-4P). When tumor vaccines are administered in the context of Treg blockade (with anti-CD25 antibody), the anti-tumor effect is heightened. Our previous work also demonstrated that the inclusion of CD137L in the vaccine preparation is uniquely responsible for a strong anti-tumor T cell response. However, when we switched from tumor-challenge to tumor-bearing regimens the vaccine was ineffective. Even the inclusion of gene expression vectors encoding GM-CSF, IL-15, lymphotactin, or SLC did not inhibit tumor progression. In order to determine if any impact could be made in tumor-bearing animals, we initiated a tumor-bearing model system featuring HSCT. Analysis of lymphoid reconstitution post-HSCT revealed that up to day 21, mice remained severely lymphopenic. However, it was during this time period that vaccination with AGN2a-4P proved most effective in a tumor-challenge experiment. Moreover, vaccination early post-HSCT was markedly improved when T cells were adoptively transferred 3 days after HSCT, just prior to the initiation of AGN2a-4P vaccination. ELISPOT data with purified CD4 and CD8 cells indicated that adoptively transferred lymphocytes early post-conditioning generated an IFN-γ-producing tumor-specific effector population. Furthermore, splenic reconstitution studies at day 21 clearly indicated that animals given lymphocyte adoptive transfer had greater percentages of donor (as opposed to residual host) T cells in their spleens. This indicates that combining adoptive T cell transfer with HSCT alters the lymphocyte populations present early post-HSCT, and that these populations are crucial to the generation of anti-tumor effector cells. We then tested our post-HSCT vaccine strategy in a tumor-bearing model. 1×10^5 live tumor cells were given on day -8, TBI on day -1, HSCT consisting of bone marrow plus 6×10^6 T cells on day 0, and irradiated AGN2a-4P vaccine on days 2 and 7. Effective neuroblastoma therapy required HSCT, T cell transfer and AGN2a-4P vaccination. Our results support the current practice of autologous HSCT for neuroblastoma therapy, and suggest that the addition of vaccination and adoptive immunotherapy (T cell transfer) to these regimens may improve outcomes.

73

LOW RATES OF TOXICITY AND LONG-TERM RESPONSES AFTER BU/FLU/ATG RIC ALLOGENEIC TRANSPLANTATION IN VERY HIGH RISK PEDIATRIC PATIENTS INELIGIBLE FOR MYELABLATIVE THERAPY: A PEDIATRIC BLOOD AND MARROW TRANSPLANT CONSORTIUM STUDY

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The role of reduced intensity conditioning (RIC) regimens in pediatric pts is unclear. To define the feasibility and toxicity of this approach using multiple stem cell sources in pediatric pts ineligible for myeloablative transplant, we conducted a trial at 25 centers participating in the Pediatric Blood and Marrow Transplant Consortium (PBMTC). Thirty two pediatric pts (age 2-20yrs, med 12) have been enrolled to date with the following stem cell sources: 7 related donor (RD) BM (2 mismatched); 5 RD-PBSC; 9 unrelated donor (UD) BM; 4UD-PBSC; 7 UD-CB. Qualifying indications included a previous allogenic (n=15) or autologous (n=4) transplant, severe organ toxicity (n=5), invasive fungal infection (n=2), and other comorbidities, (n= 6 pts, 4 with Down syndrome). Diagnoses included ALL (4 CR2; 9 CR3), AML (6 CR2; 3 CR3; 1 secondary), MLL (1 CR3), HD (2 CR3), B-NHL (1 PR3), MDS (2 RA;